

Construction of JB-3120-2, a plasmid containing T7 H91A *hin47* (+ leader)

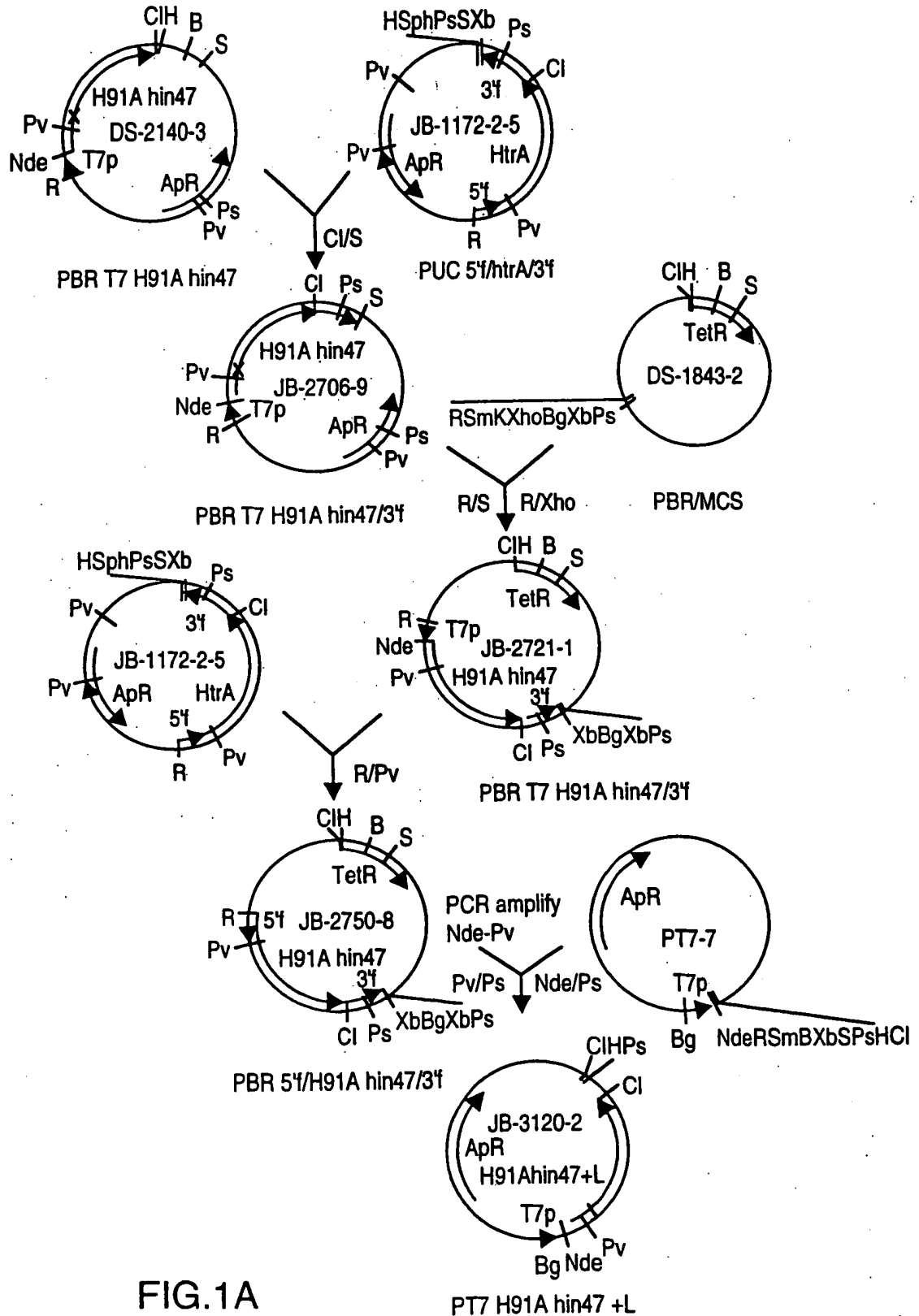


FIG.1A

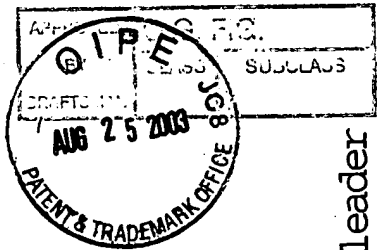
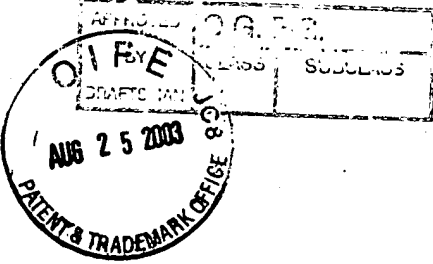


FIG.1B

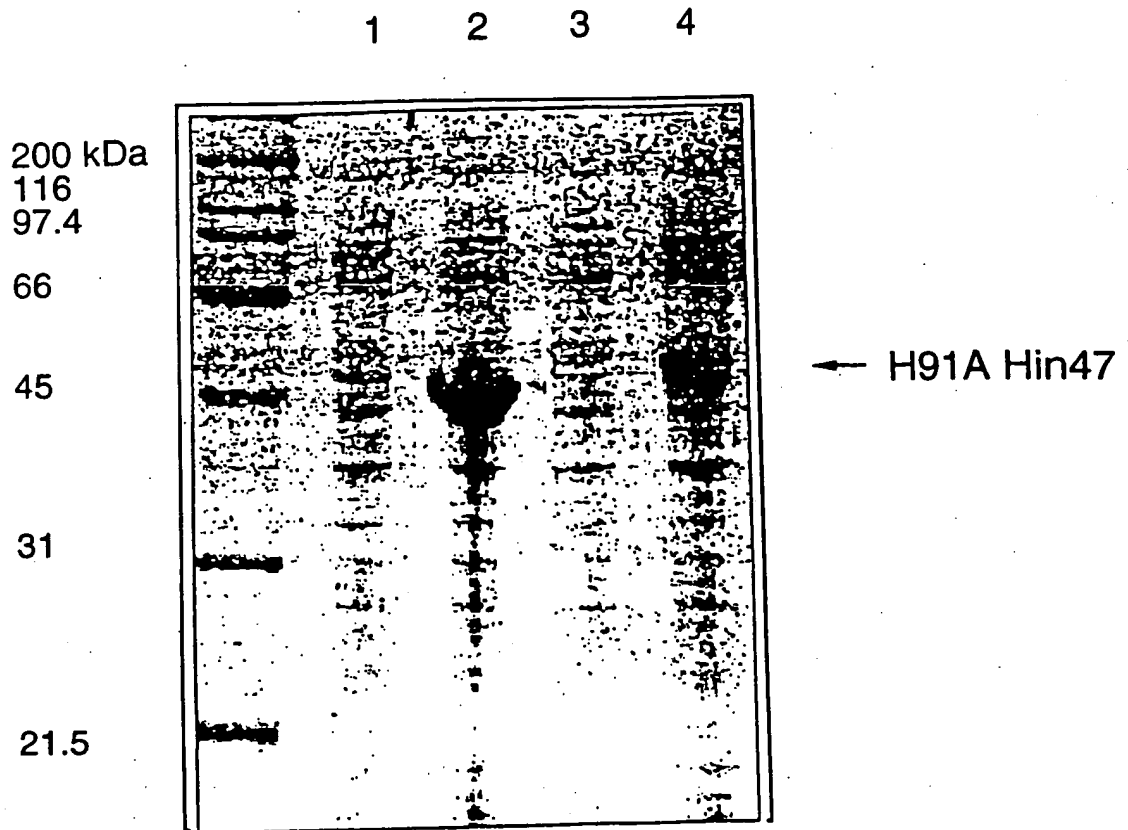
Oligonucleotide primers to PCR amplify the Nde I-Pvu I fragment encoding the leader sequence of HtrA.

Nde I			
	M K K T R F V L N S		SEQ ID No.2
5'	GGCGCATATCAAAAAACACGTTTGTACTAAATAGT	3' 6931.SL	SEQ ID No.1

Pvu I			
	F K F F F G D R F A E Q		SEQ ID No.5
	TTTAAATCTCTTTCGCGATCGTTTTCGCCGAACAA		SEQ ID No.4
3'	AAATTTAAGAACAAACCGCTAGCAAAACCGCTTGT	5' 6932.SL	SEQ ID No.3

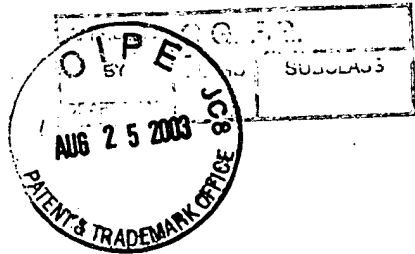


# Production of H91A Hin47 +/- leader



- 1. H91A Hin47 (- leader)  $t_0$
- 2. H91A Hin47 (- leader)  $t_4$
- 3. H91A Hin47 (+ leader)  $t_0$
- 4. H91A Hin47 (+ leader)  $t_4$

FIG.2



# Purification of H91A Hin47 (+ leader) from *E. coli*

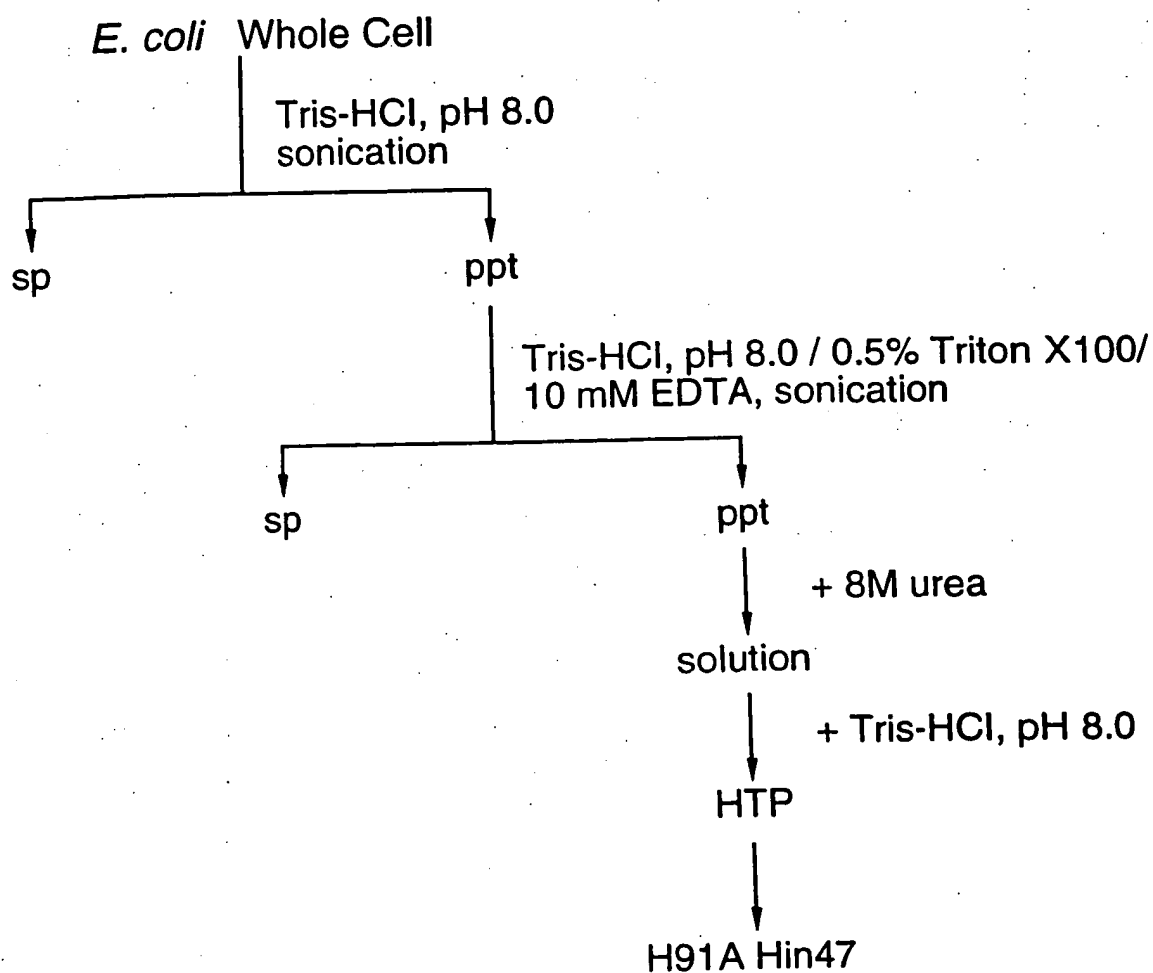
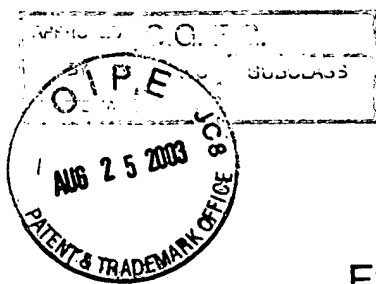


FIG.3



# Extraction of H91A Hin47 +/- leader, produced in *E. coli*

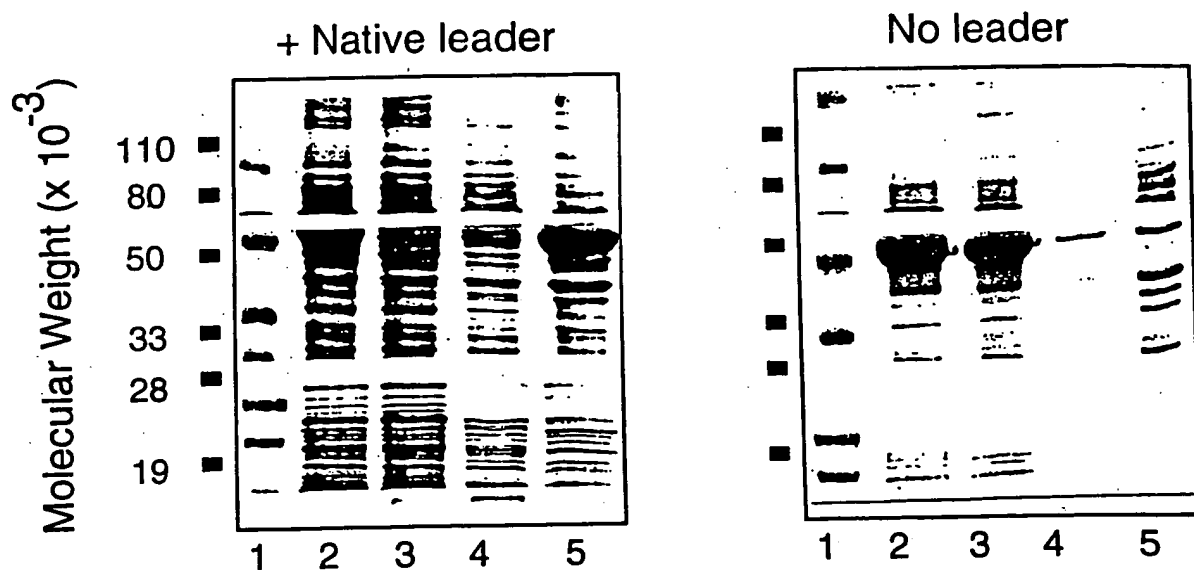
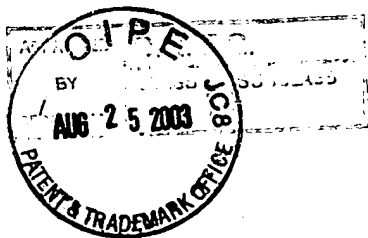


FIG.4



Construction of DS-2342-2-2, a plasmid containing T7 H91A *hin47* and T7 V38 *hia*

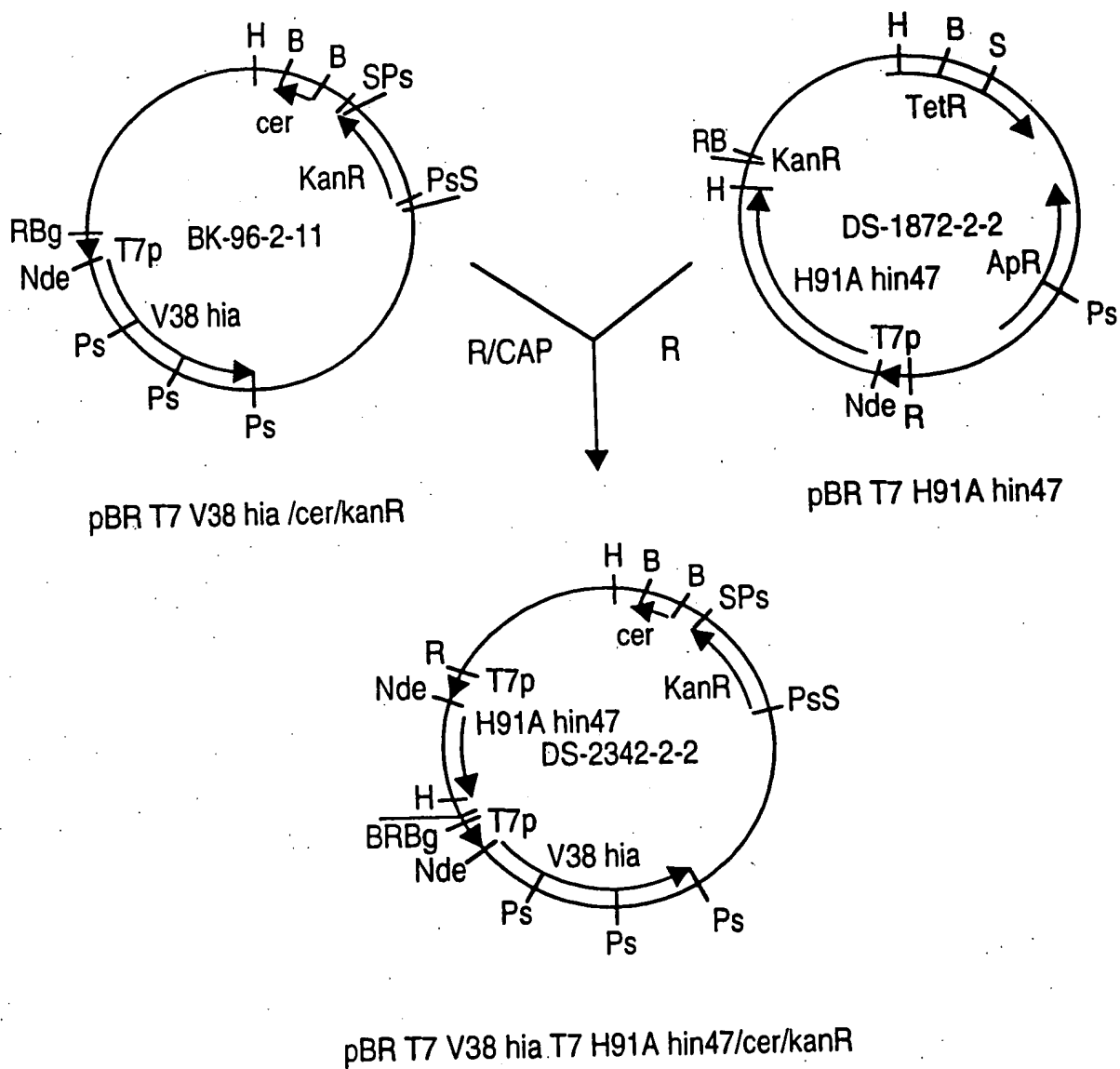
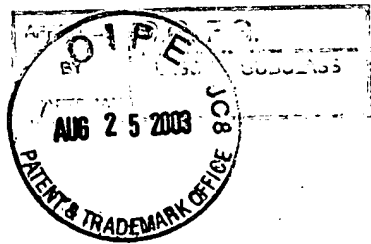
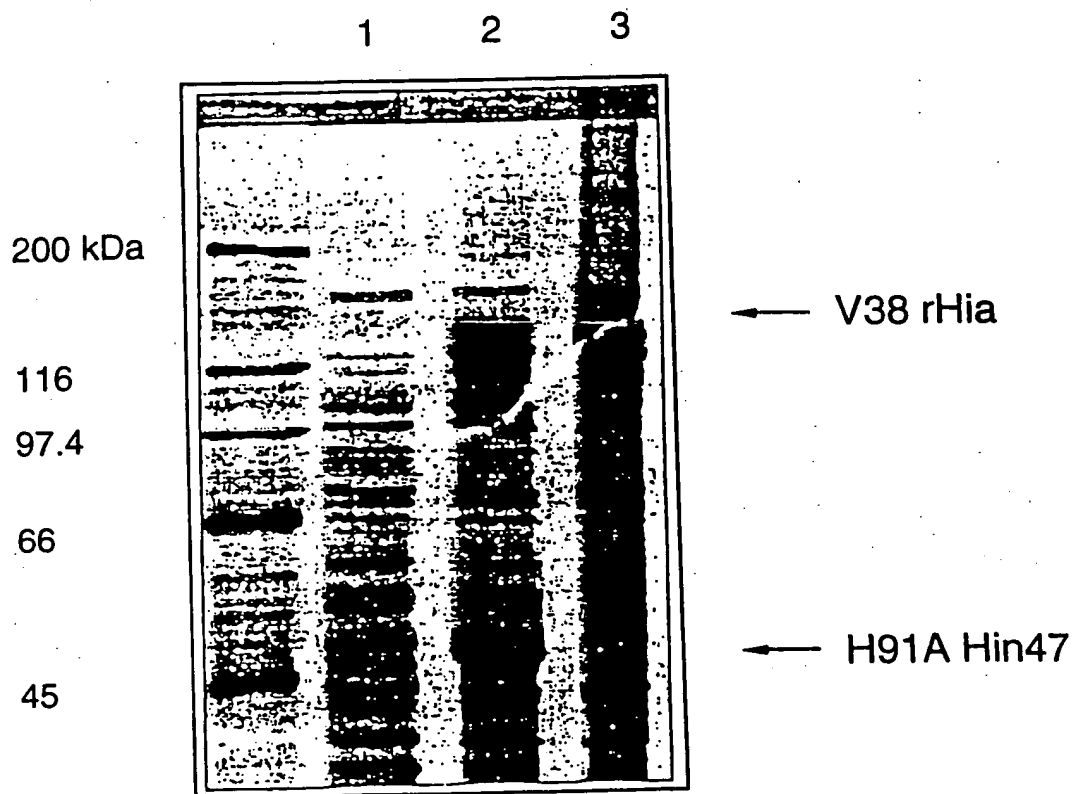


FIG.5

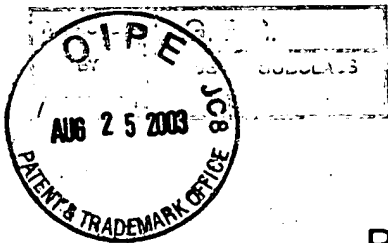


# Production of V38 rHia and H91A Hin47 when co-expressed



1. V38 rHia and H91A Hin47  $t_0$
2. V38 rHia and H91A Hin47  $t_4$
3. V38 rHia alone  $t_4$

FIG.6



# Purification of H91A Hin47 and V38 rHia from *E. coli*

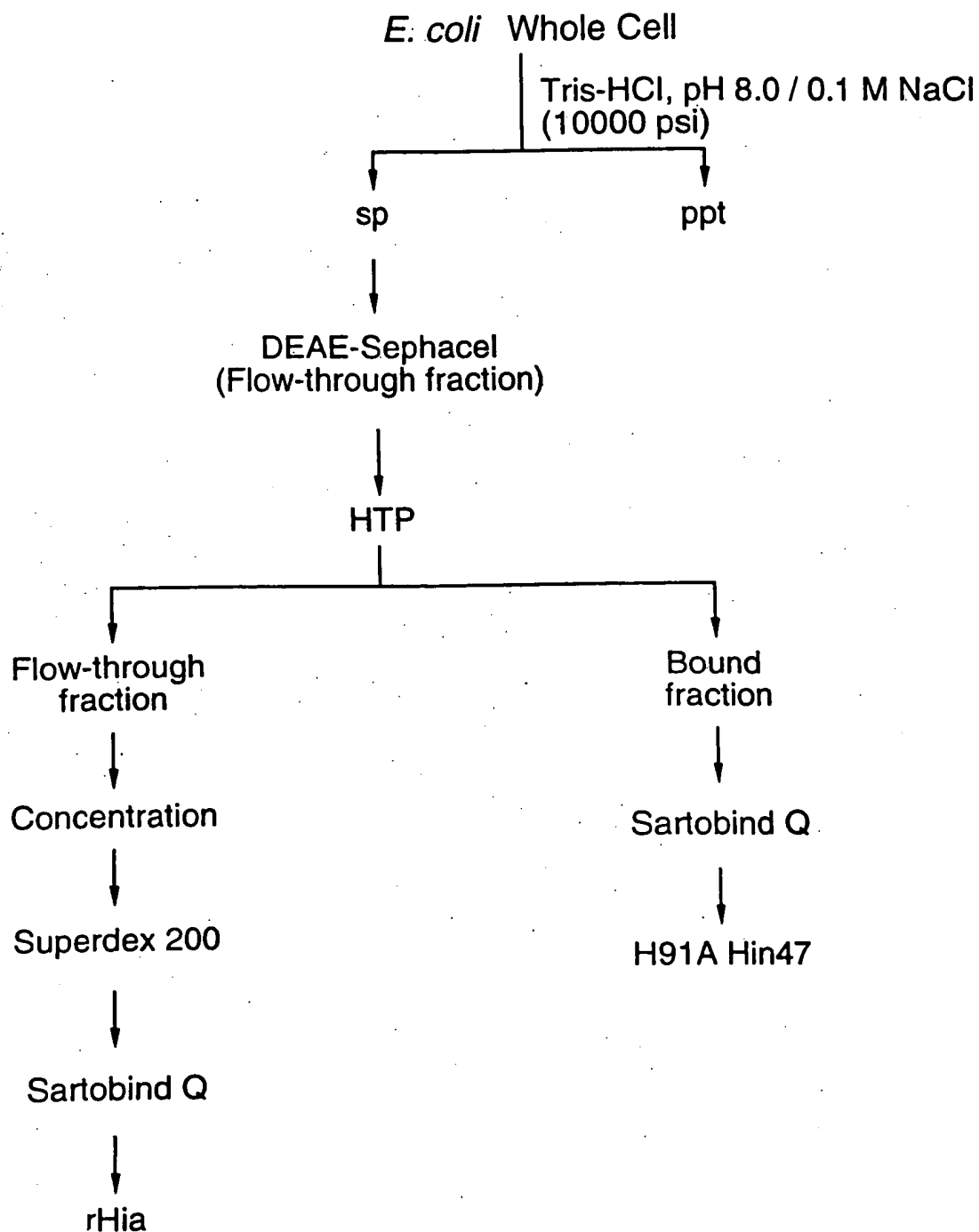
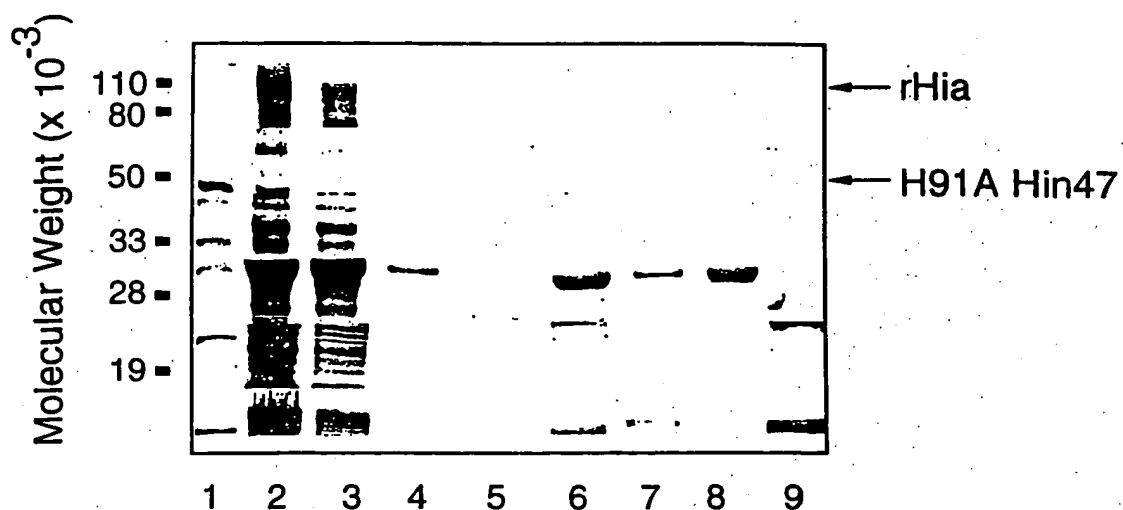


FIG.7





Purification of H91A Hin47 and V38 rHia from *E. coli*



- |  |                        |
|--|------------------------|
| 1. Prestained molecular weight markers | 6. DEAE flow-through   |
| 2. <i>E. coli</i> whole cell lysate    | 7. HTP flow-through    |
| 3. Tris/NaCl extract                   | 8. Purified H91A Hin47 |
| 4. Tris/ Triton X-100 / EDTA extract   | 9. Purified rHia       |
| 5. Tris/ OG extract                    |                        |

FIG.8

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Construction of JB-3134-1-1, a plasmid containing T7 H91A hin47 (-leader) and T7 S44 hia

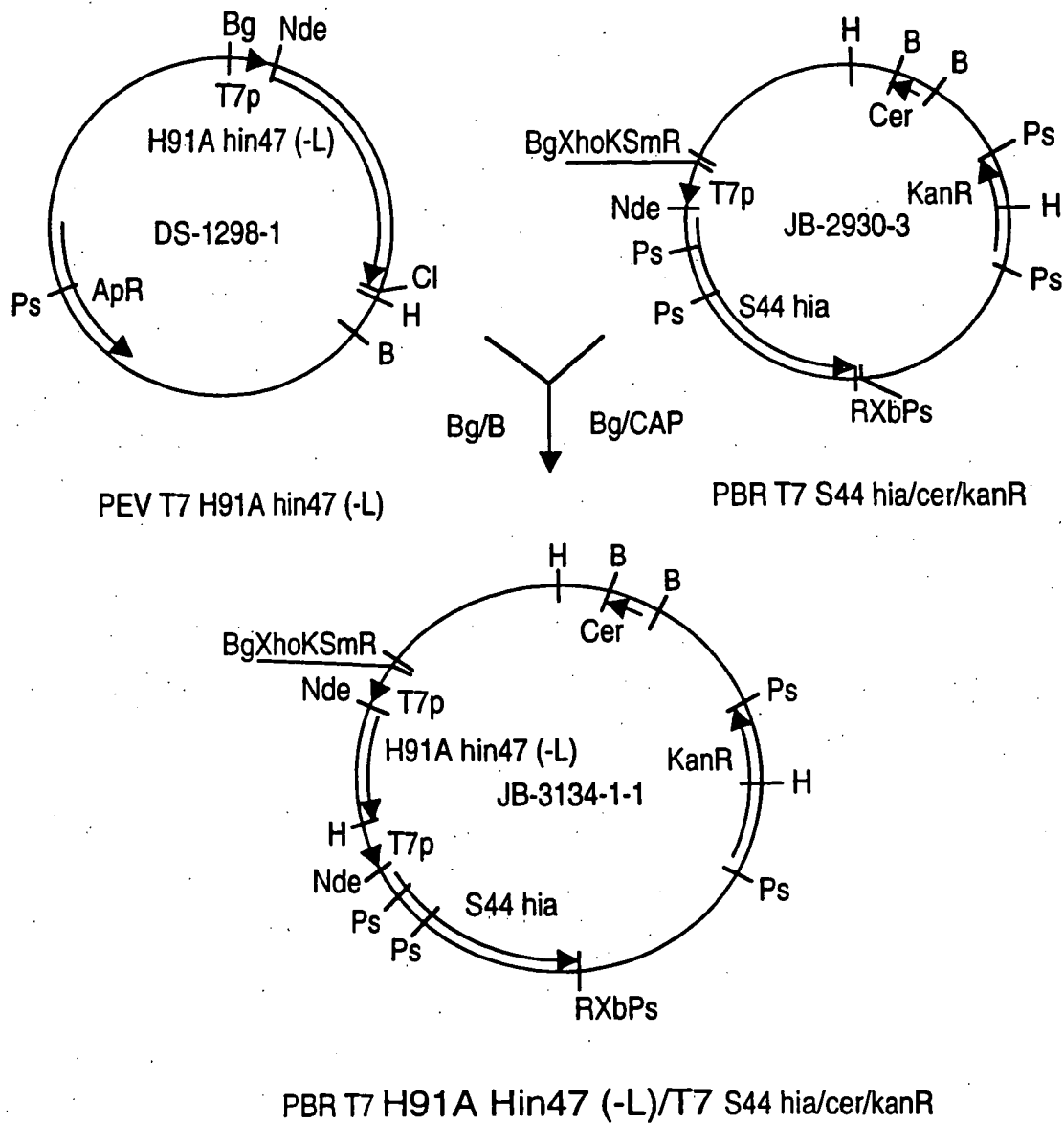
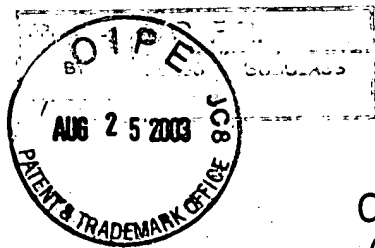


FIG.9



Construction of JB-3145-1, a plasmid containing T7 H91A hin47  
(+ leader) and T7 S44 hia

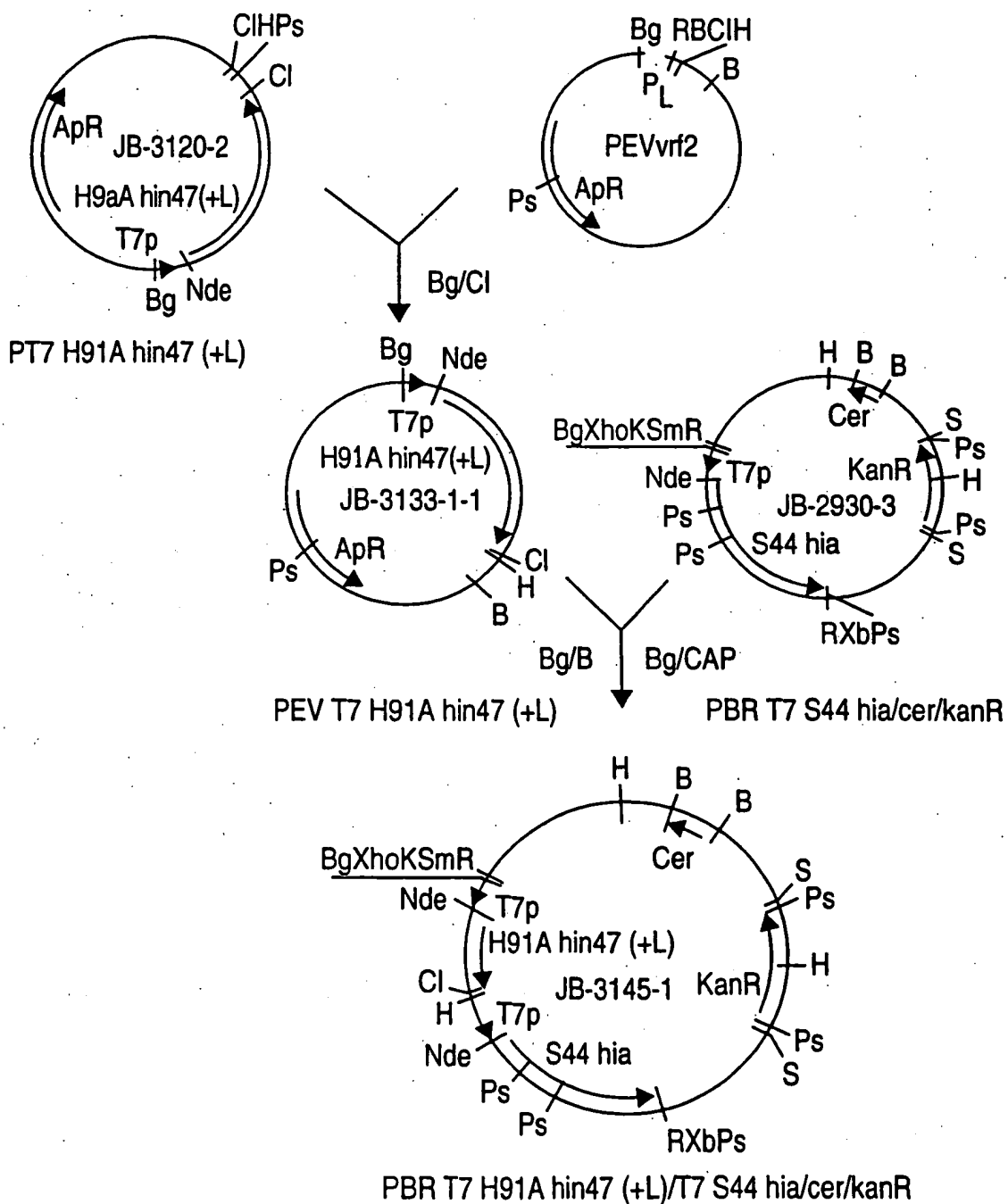
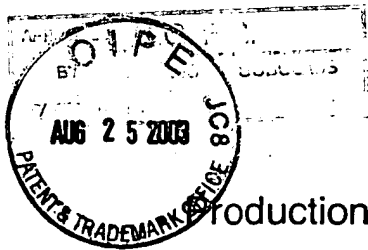
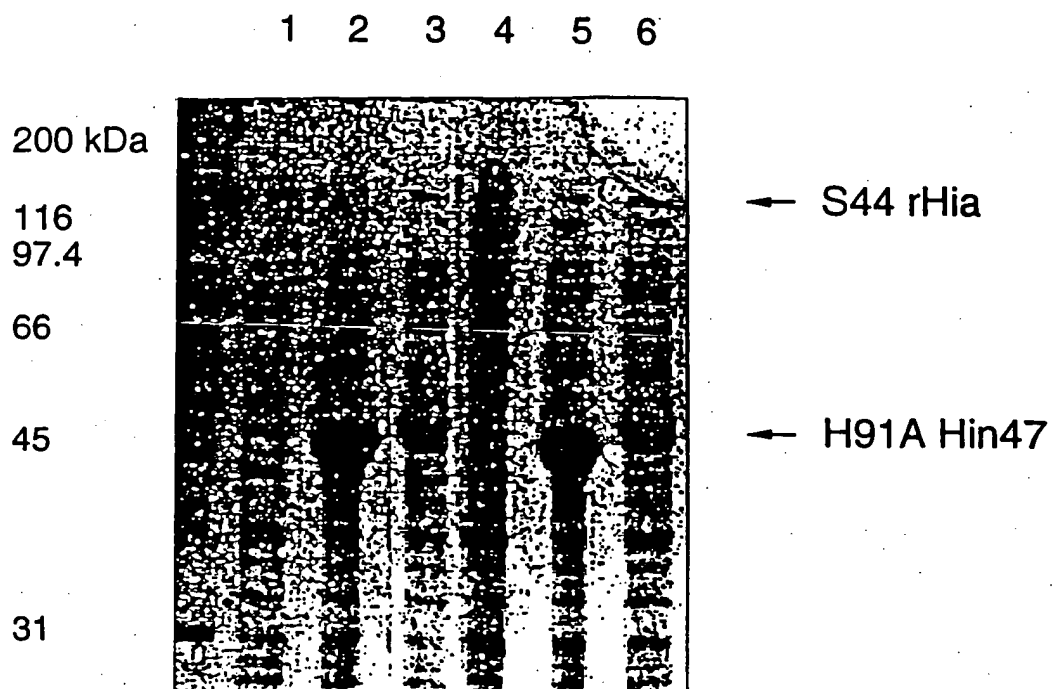


FIG.10



Production of S44 rHia and H91A Hin47 (+/-L), when co-expressed.



- |                               |                |
|-------------------------------|----------------|
| 1. H91A Hin47 (-L)            | t <sub>0</sub> |
| 2. H91A Hin47 (-L)            | t <sub>4</sub> |
| 3. H91A Hin47 (+L)            | t <sub>4</sub> |
| 4. S44 rHia                   | t <sub>4</sub> |
| 5. H91A Hin47 (-L) + S44 rHia | t <sub>4</sub> |
| 6. H91A Hin47 (+L) + S44 rHia | t <sub>4</sub> |

FIG.11



Construction of JB-3073R-1, a plasmid containing T7 *psaA* (+ leader) and T7 H91A *hin47*

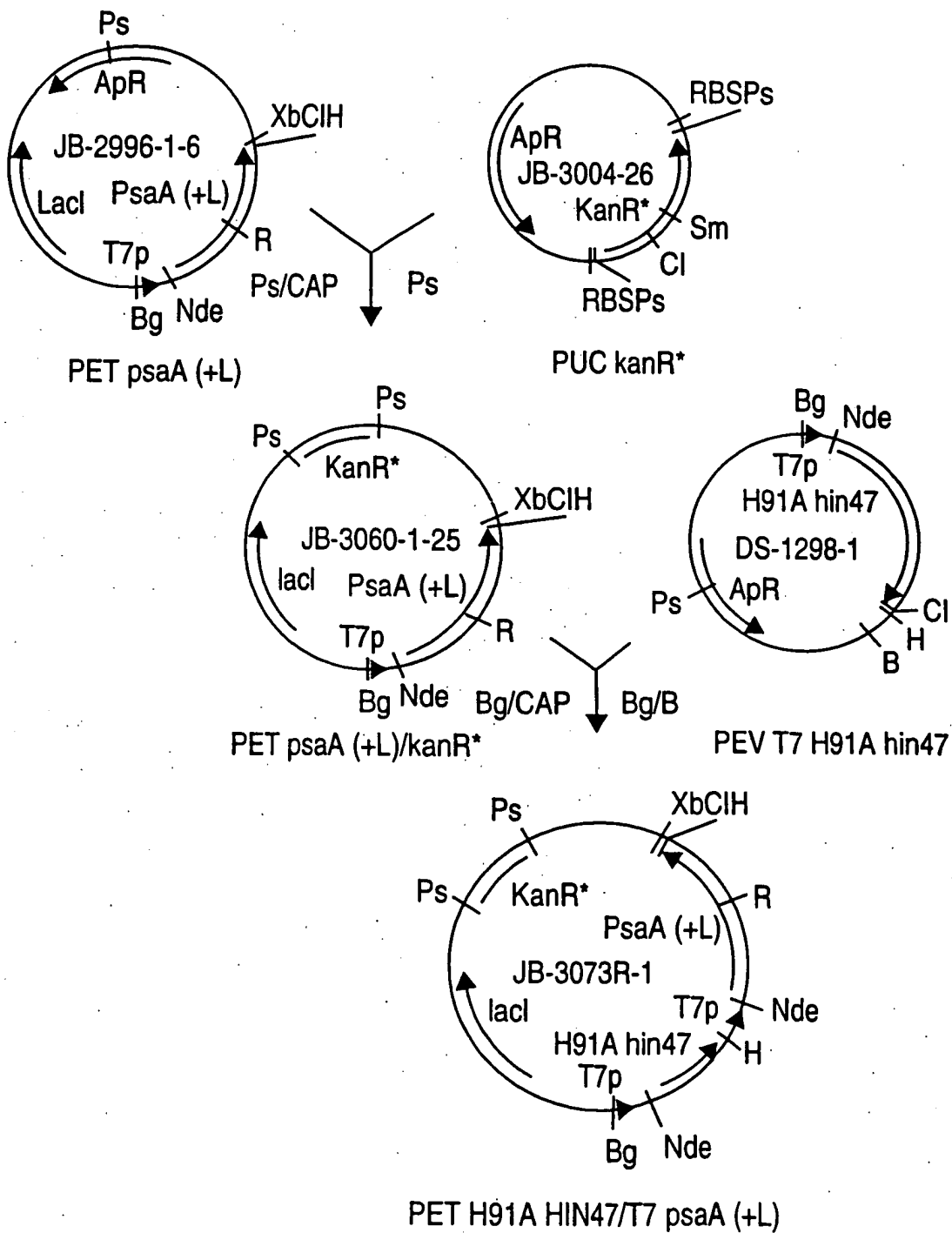
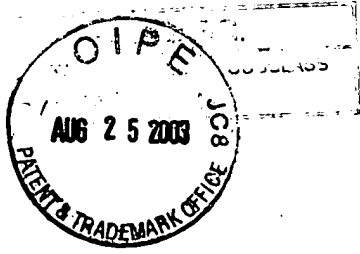


FIG.12A



## FIG.12B

Oligonucleotide primers for PCR amplify psaA(+native leader)

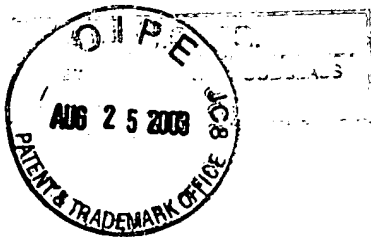
Sense:

Nde I

5' CCGCATCC CATATG M K K L G T L L V L 3' 6850.SL SEQ ID No.27  
CATATG AAAAAATTAGTACATTACTGTTCTC 3' 6850.SL SEQ ID No.26

Antisense:

3' CATGTGAGCTGGCGTTT TTTAAAGCTTGGCC 5' 6852.SL SEQ ID No.29  
GTACACTGACCGCAAAAAA TTTCGAA CCGG 5' 6852.SL SEQ ID No.28  
Hind III



Construction of JB-3090-1 and JB-3090-7, plasmids containing  
T7 psaA (-leader) and T7 H91A hin47

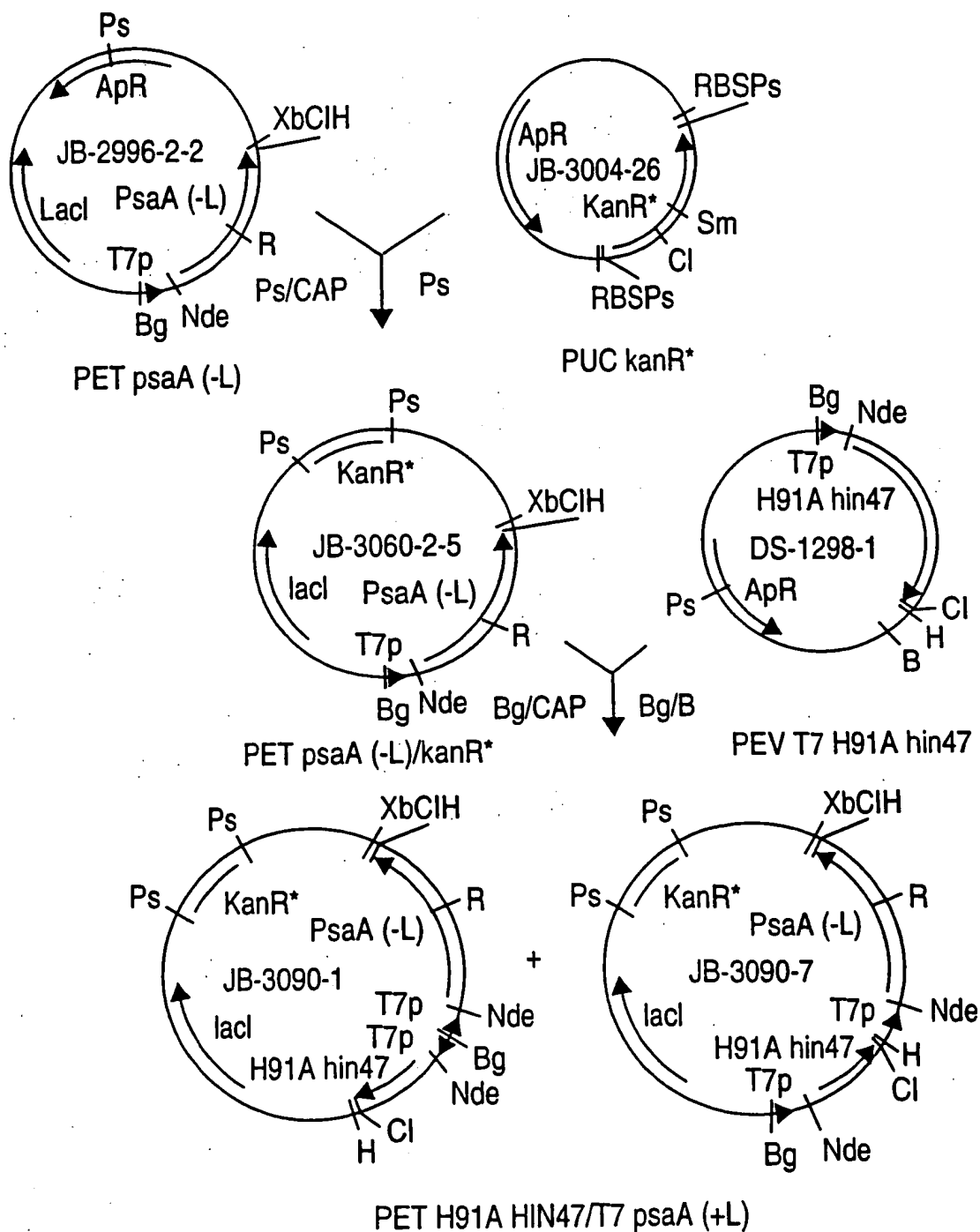
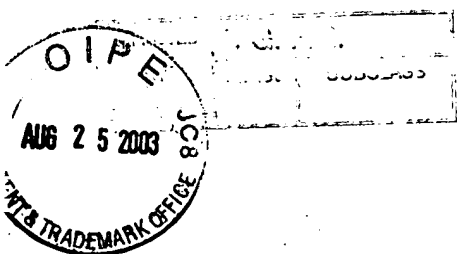


FIG.13A



# FIG.13B

Oligonucleotide primers to PCR amplify psaA (- leader)

Sense:

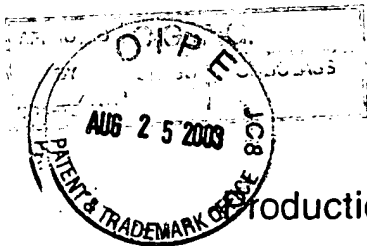
	M	C	A	S	G	K	K	D	T			
5'	CGG	CAT	CC	CAT	ATG	IGT	CTAG	CGG	AAAAA	AG	ATACA	3'
												6851.SL
												SEQ ID No.31
												SEQ ID No.30

Antisense:

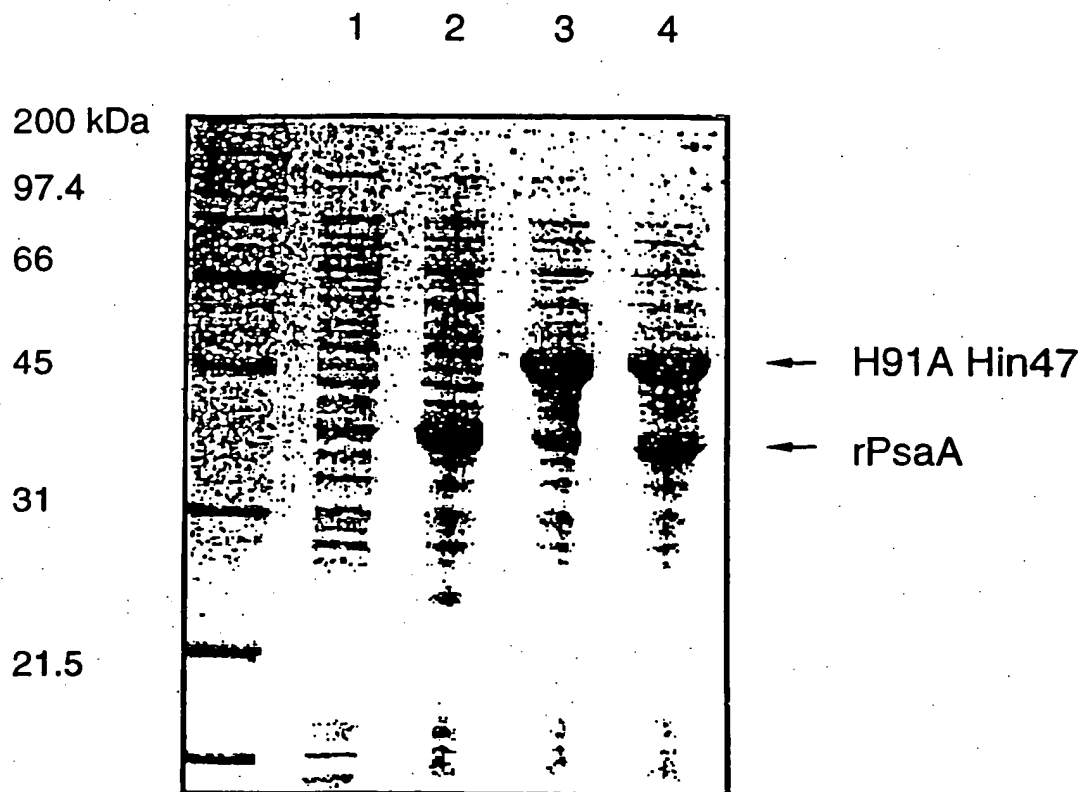
	CAT	GIG	AGC	TGG	GT	TTT	TAA	GCT	TGG	CC		
3'	GTAC	ACT	CG	ACCG	CA	AAAAA	TT	CGA	ACCG			
												5'
												6852.SL
												SEQ ID No.29
												SEQ ID No.28

Hind III



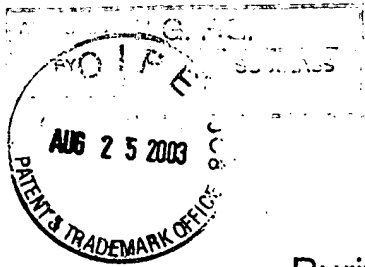


roduction of rPsaA +/- leader and H91A Hin47, when co-expressed.



1. H91A Hin47 and rPsaA (+ leader), < > orientation t<sub>0</sub>
2. H91A Hin47 and rPsaA (+ leader), < > orientation t<sub>4</sub>
3. H91A Hin47 and rPsaA (+ leader), > > orientation t<sub>4</sub>
4. H91A Hin47 and rPsaA (- leader), > > orientation t<sub>4</sub>

FIG.14



# Purification of H91A Hin47 and rPsaA from *E. coli*

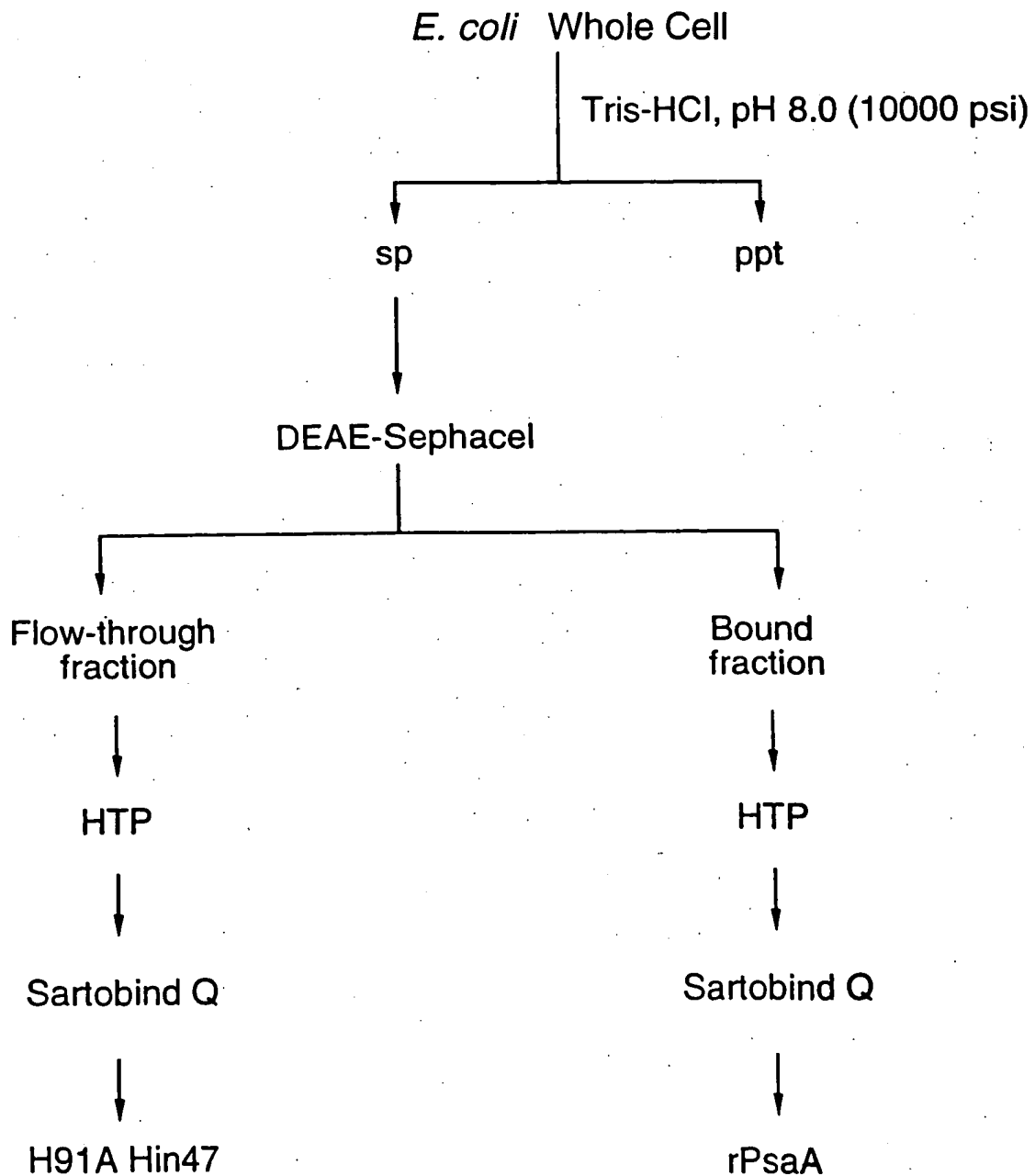
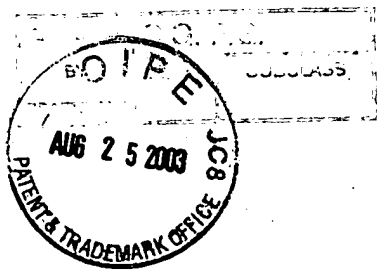
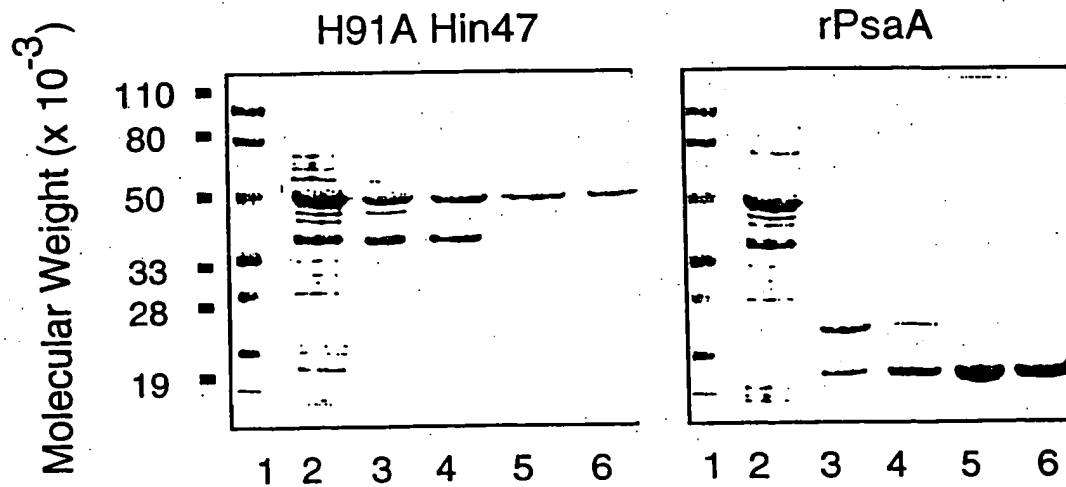


FIG.15



# Purification of H91A Hin47 and rPsaA from *E. coli*



1. Pre-stained molecular weight markers
2. *E. coli* whole cell lysate
3. Soluble proteins in 50mM Tris-HCl, pH 8.0 extraction
4. DEAE-Sepacel column
5. HTP column
6. Sartobind Q membrane

FIG.16

# Construction of a plasmid containing T7 hmwBC

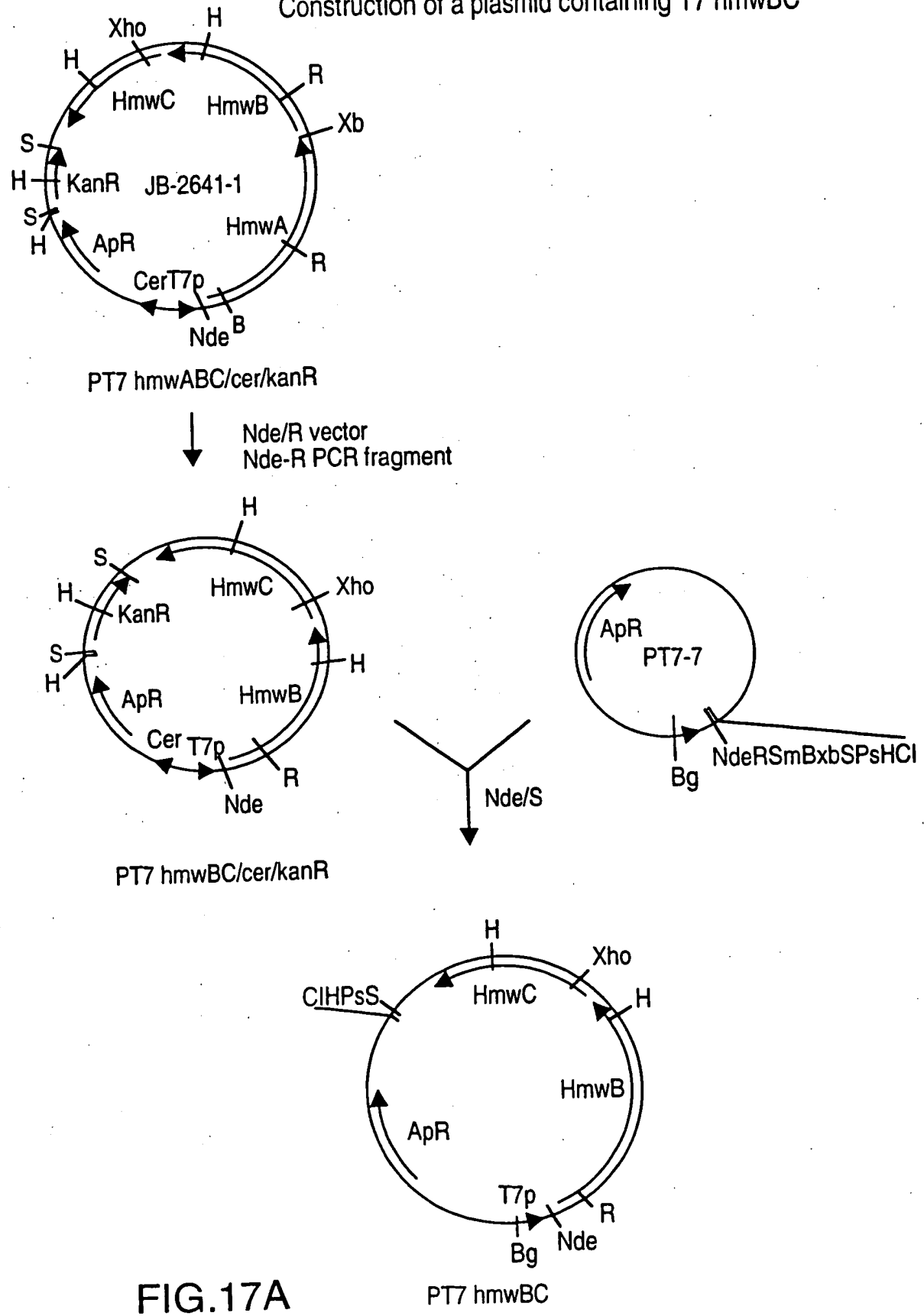


FIG.17A

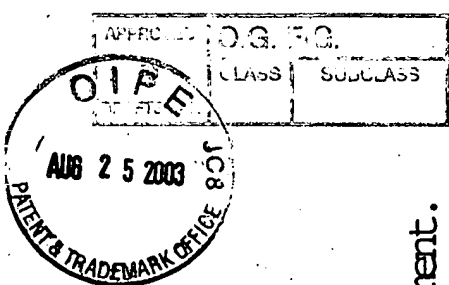


FIG.17B

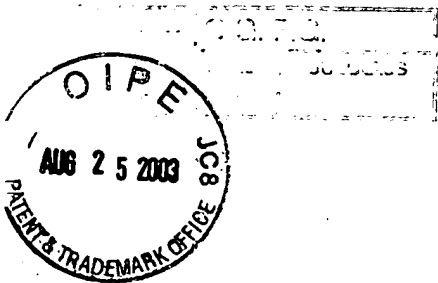
Oligonucleotide primers for PCR amplification of Nde I-EcoR I 5' *hmmB* fragment.

coding strand:

Nde I		
	M K N I K S R L K L	
5'	GGCGC <u>CATATG</u> <u>AAAAATATATAAA</u> ACCAGATTA AACTC	SEQ ID No:7
	3' 7072..SL	SEQ ID No:6

non-coding strand:

EcoR I		
	G R Q W F D L R E F N M A	
	GGTCGTCAGTCGGTTCGATTTCGGT <u>CAATTCA</u> ATATGGCA	
3'	CCAGCAGTCACCAAGCTAAACGCACCTTAAGTTATACCGT	SEQ ID No:10
	5' 5950..SL	SEQ ID No:9
		SEQ ID No:8



# Construction of plasmid containing T7 hmwB

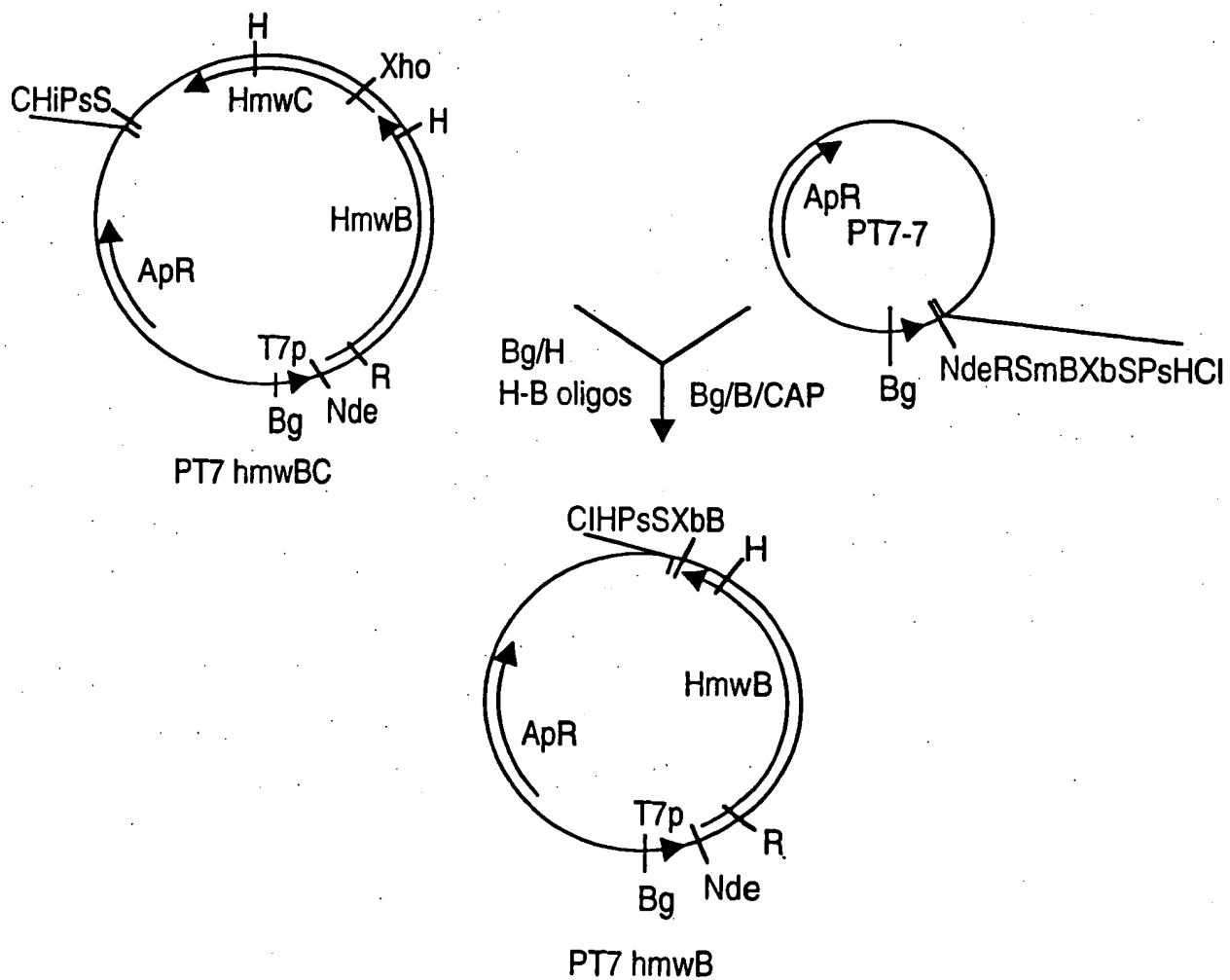


FIG.18A



FIG.18B

Oligonucleotides for construction of the Hind III-BamH I 3' *hmvB* fragment.

Hind III

S L D A F V A R R F A N A N S D N L N G N K K R T S S P T T...  
AGCT TAGATGCTTTTGTGTGCTGCTTTCGAATGCCAATAGTACACATTTGAATGCCACAAAAA

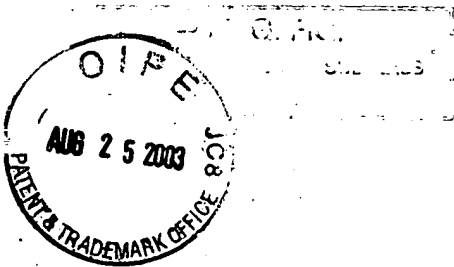
... F W G R L T F S F \* \*  
...  
... CTTCTGGGGTAGATTAAACATTCAGTTTCTAATAG  
CCACAAAGCTCACTACAAC...  
SEQ ID No:13  
7073.SL  
SEQ ID No:11  
7074.SL  
SEQ ID No:12

ATCTACGAAACAACGACGACGCGAAACGTTACGGTTATCACTGTAAACTTACCGTTGTTTGGGTGTTCGAG

...  
TCGATGTGTG...

...  
... GAAGACCCCATCTAATTGTAAAGTAAAGATTATCTAG  
...  
7076.SL  
7075.SL  
SEQ ID No:15  
SEQ ID No:14

BamH I



### Construction of a plasmid containing T7 hmwC

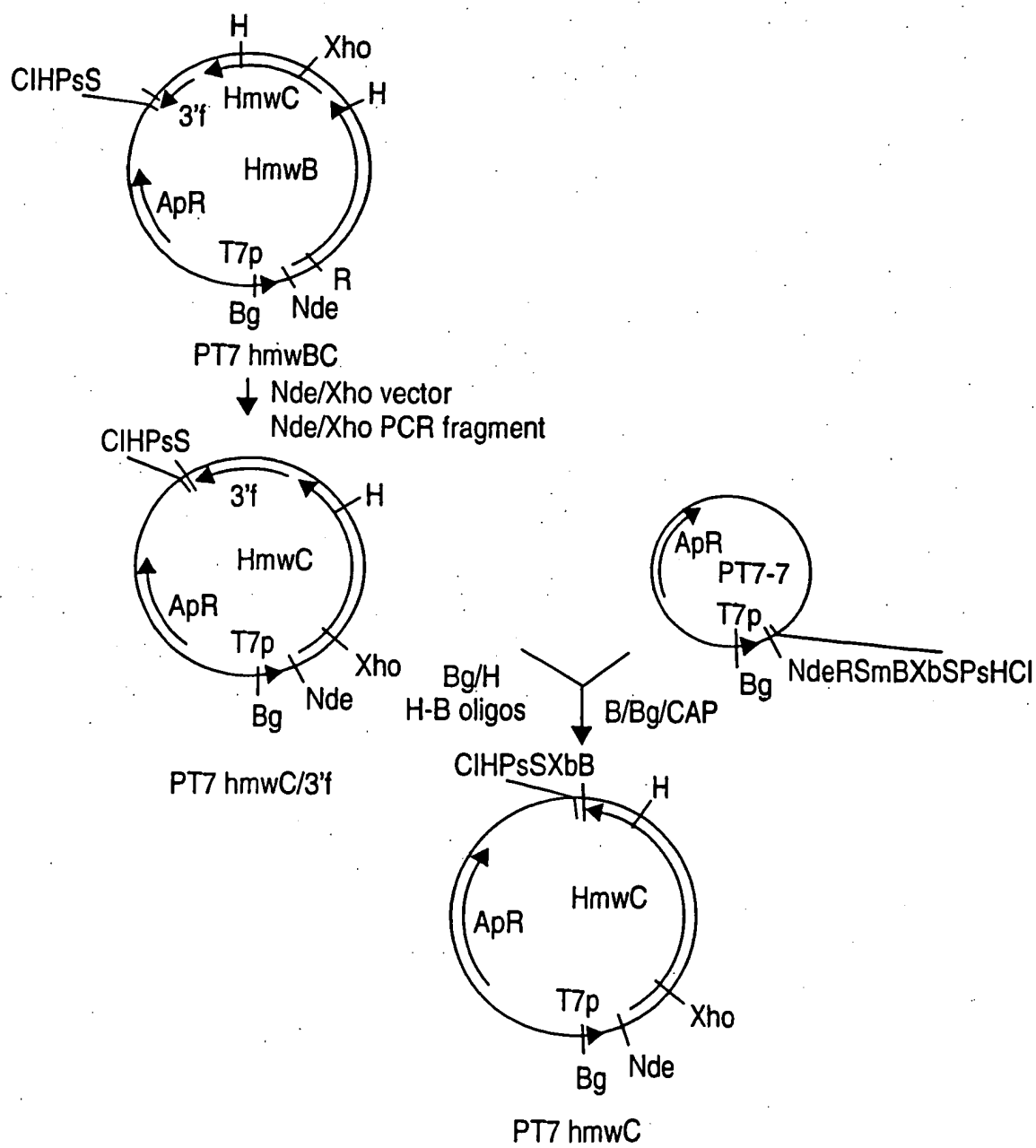


FIG.19A



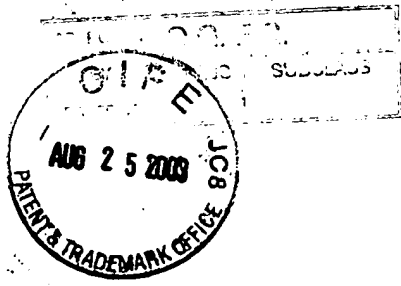


FIG.19B

Oligonucleotide primers for PCR amplification of Nde I-Xho I 5' hmwC fragment.

coding strand:

Nde I			
	M T K E N L Q S V P		SEQ ID No:17
5'	GGCCCATATGACAAAGAAANTTACAAAGIGITCCA	3' 7077.SL	SEQ ID No:16

non-coding strand:

Xho I			
	S T S M I A A R E K F Y		SEQ ID No:20
	TCAACTTCAATGATTCCTCTCGACAAAATTCAT		SEQ ID No:19
3'	AGTTGAAGTTACTAACGACGAGCTCTTTTAAAGATA	5' 7078.SL	SEQ ID No:18

**FIG. 19C**

Oligonucleotides for construction of Hind III-BamH I 3' hmwC fragment.

### Hind III

L F T G D P R P L G K I L L K K T N E W...

AGCTTTTATACGGGCAACCTGGTCCATTGGCGAAATA

CTGCTTAAGAAACAAATCAATG...

SEO ID No: 23

7079.SL SEO ID No:21

7080.SL SEQ ID No:22

...GAAGCGAAGCAGCTTCAGTAAATAATATAG

AAATGTCGGTCGGAGCAGTAACCGTTTATGACCAATTC

TTTGGTTACTTAC...

7082.SL SEQ ID No:25

7081.SL SEQ ID No:24

...  
CTTTCACCTCAGGCGAATTTTATTATCTCTAG

## Barth I